

What is claimed is:

Rule B1

1. An antibody-based fusion protein comprising an immunoglobulin (Ig) chain linked to a non-Ig protein via a junction point, wherein said antibody-based fusion protein comprises an amino acid alteration within 10 amino acids from said junction point, in said Ig chain or said non-Ig protein, and wherein said antibody-based fusion protein has a longer circulating half-life *in vivo* than a corresponding antibody-based fusion protein without said amino acid alteration.
2. The fusion protein of claim 1 wherein the amino acid alteration increases the hydrophobicity of said antibody-based fusion protein.
3. The fusion protein of claim 1 or 2 wherein said Ig chain is N-terminal to said non-Ig protein.
4. The fusion protein of claim 1, 2 or 3 wherein said alteration changes the C-terminal amino acid of the Ig chain.
5. The fusion protein of claim 1, wherein said non-Ig protein is a secreted protein.
6. The fusion protein of claim 5, wherein said non-Ig protein is a mature form of said secreted protein.
7. The fusion protein of claim 1, wherein the Ig chain comprises part of an Ig heavy chain.
8. The antibody-based fusion protein of claim 7 wherein said Ig chain comprises at least the CH2 domain of an IgG2 or an IgG4 constant region.
9. The antibody-based fusion protein of claim 7, wherein said Ig chain comprises at least a portion of an IgG1 constant region having a mutation or a deletion at one or more amino acids selected from the group consisting of Leu₂₃₄, Leu₂₃₅, Gly₂₃₆, Gly₂₃₇, Asn₂₉₇, and Pro₃₃₁.
10. The antibody-based fusion protein of claim 7, wherein said Ig chain comprises at least a portion of an IgG3 constant region having a mutation or a deletion at one or more amino acids selected from the group consisting of Leu₂₈₁, Leu₂₈₂, Gly₂₈₃, Gly₂₈₄, Asn₃₄₄, and Pro₃₇₈.

Rule B2

These are the claims

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11. The antibody-based fusion protein of claim 7, wherein said Ig chain has binding affinity for an immunoglobulin protection receptor.
12. The antibody-based fusion protein of claim 7, wherein said Ig chain has substantially reduced binding affinity for a Fc receptor selected from the group consisting of Fc γ RI, Fc γ RII and Fc γ RIII.
13. The antibody-based fusion protein of claim 7, wherein said non-Ig protein is selected from the group consisting of a cytokine, a ligand-binding protein, and a protein toxin.
14. The antibody-based fusion protein of claim 13, wherein said cytokine is selected from the group consisting of a tumor necrosis factor, an interleukin, and a lymphokine.
15. The antibody-based fusion protein of claim 14, wherein said tumor necrosis factor is tumor necrosis factor alpha.
16. The antibody-based fusion protein of claim 14, wherein said interleukin is interleukin-2.
17. The antibody-based fusion protein of claim 14, wherein said lymphokine is a lymphotoxin or a colony stimulating factor.
18. The antibody-based fusion protein of claim 11, wherein said colony stimulating factor is a granulocyte-macrophage colony stimulating factor.
19. The antibody-based fusion protein of claim 13, wherein said ligand-binding protein is selected from the group consisting of CD4, CTLA-4, TNF receptor, and an interleukin receptor.
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20. A method for increasing the circulating half-life of an antibody-based fusion protein having an Ig chain linked to a non-Ig protein via a junction point, the method comprising the step of substituting, deleting, inserting, or otherwise altering an amino acid at or near said junction point.
21. The method of claim 20, wherein said fusion protein comprises a portion of a heavy chain.

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22. The method of claim 21, wherein said fusion protein comprises at least the CH2 domain of an IgG2 or an IgG4 constant region.

23. The method of claim 20, 21, or 22, wherein said fusion protein comprises a heavy chain moiety having a mutation that affects interaction with an Fc protection receptor.

24. The fusion protein of claim 1 comprising a linker between said Ig chain and said non-Ig protein.

25. The fusion protein of claim 4, 5, 6, or 7, wherein said alteration is a substitution of one or more amino acids.

26. An antibody-based fusion protein comprising

- a) a first polypeptide comprising an Ig chain, and,
- b) a second polypeptide comprising a non-Ig protein,

wherein said first polypeptide is joined to said second polypeptide to produce a junction region having at least one mutation, and

wherein said fusion protein has a longer circulating half life than a fusion protein having a junction region without said mutation.

27. The fusion protein of claim 26, wherein said mutation is in the C-terminal portion of said first polypeptide.

28. The fusion protein of claim 26, wherein said mutation is in the N-terminal portion of said second polypeptide.

29. The fusion protein of claim 26 comprising a first mutation in the C-terminal portion of said first polypeptide and a second mutation in the N-terminal portion of said second polypeptide.

30. The fusion protein of claim 27 or 29 wherein said C-terminal portion comprises between 1 and 100 C-terminal amino acids of said first polypeptide.

31. The fusion protein of claim 30 wherein said C-terminal portion comprises between 1 and 10 C-terminal amino acids of said first polypeptide.

32. The fusion protein of claim 28 or 29 wherein said N-terminal portion comprises between 1 and 100 N-terminal amino acids of said second polypeptide.

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33. The fusion protein of claim 32 wherein said N-terminal portion comprises between 1 and 10 N-terminal amino acids of said second polypeptide.

34. The fusion protein of claim 26 wherein said Ig is IgG1.

35. The fusion protein of claim 26 wherein said mutation is selected from the group consisting of point mutations, deletions, insertions, and rearrangements.

36. The fusion protein of claim 34 wherein the C-terminal residue of said first polypeptide is mutated to be an amino acid with a non-ionizable side chain.

37. The fusion protein of claim 36 wherein said C-terminal residue is a non-lysine amino acid.

38. The fusion protein of claim 26 wherein said junction region consists of the C-terminal region of said first polypeptide and the N-terminal region of said second polypeptide, and wherein said mutation is present in one of said C-terminal and N-terminal regions.

~~39.~~ The fusion protein of claim 26 wherein said junction region comprises a spacer or linker peptide.

~~40.~~ The fusion protein of claim 39 wherein said mutation consists of the presence of a spacer or linker peptide between said first and said second polypeptides.

41. The fusion protein of claim 26 wherein said mutation is in a region that does not interact with FcR or FcRp.

42. A method for identifying a mutation that increases the circulating half life of an antibody-based fusion protein having an Ig moiety and a non-Ig moiety comprising the steps of:

- introducing a mutation in the region spanning the junction between the Ig moiety and the non-Ig moiety;
- comparing the serum half-lives of the antibody-based fusion protein with and without a mutation; and,
- selecting a mutation that increases the serum half-life of the antibody-based fusion protein.

Priority

- 43. An antibody-based fusion protein comprising a mutation identified according to the method of claim 42.
- 44. A method for treating a disease comprising the step of administering to a patient an antibody-based fusion protein of claim 26.
- 45. The fusion protein of claim 1, 26, or 36, having a hydrophobic or non-polar amino acid introduced via addition or substitution at or near said junction.
- 46. The fusion protein of claim 45, wherein said amino acid is selected from the group consisting of Leu, Ala, Trp, and Gly.
- 47. The fusion protein of claim 46, wherein said amino acid is Ala.